Adverse Outcome Pathways and Skin Sensitization Testing

Warren Casey, PhD, DABT
Director, NICEATM
National Institute of Environmental Health Sciences

NTP Board of Scientific Counselors Meeting
June 17 - 18, 2014

NICEATM

NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM), organized as an office under the NTP Division, part of NIEHS

- Focus is on”3R” methods

National Toxicology Program
NTP

ehp ENVIRONMENTAL HEALTH PERSPECTIVES

15 Years Out: Reinventing ICCVAM

February 1, 2013 Editorials Comments Off

Linda S. Birnbaum

Director, NIEHS and NTP, National Institutes of Health, Department of Health and Human Services, Research Triangle Park, North

"Toxicology testing is shifting from a primary focus on adverse phenotypic observations in animals to mechanism-based biological outcomes in vitro, and the NIEHS is embracing this paradigm shift through its participation in the multiagency Tox21 consortium (Collins et al. 2008). NICEATM will expand its scope and concentrate its resources on providing bioinformatic and computational toxicology support to NIEHS Tox21 projects."

Exploring New Paradigms

- Federal agencies have recognized the need for an evolving concept of validation that is responsive to new technologies and on-going paradigm shifts in toxicity testing
- Evaluate predictive, integrated test strategies that combine *in silico* approaches, multiple *in vitro* assays, and use of other alternative systems

Adverse Outcome Pathways (AOPs)

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- *"An analytical construct that describes a sequential chain of causally linked events at different levels of biological organisation that lead to an **adverse** health or ecotoxicological effect."**
- AOPs are the central element of a toxicological knowledge framework being built to support chemical risk assessment based on mechanistic reasoning.

*OECD GD138 Developing and Assessing Adverse Outcome Pathways (April 2013)

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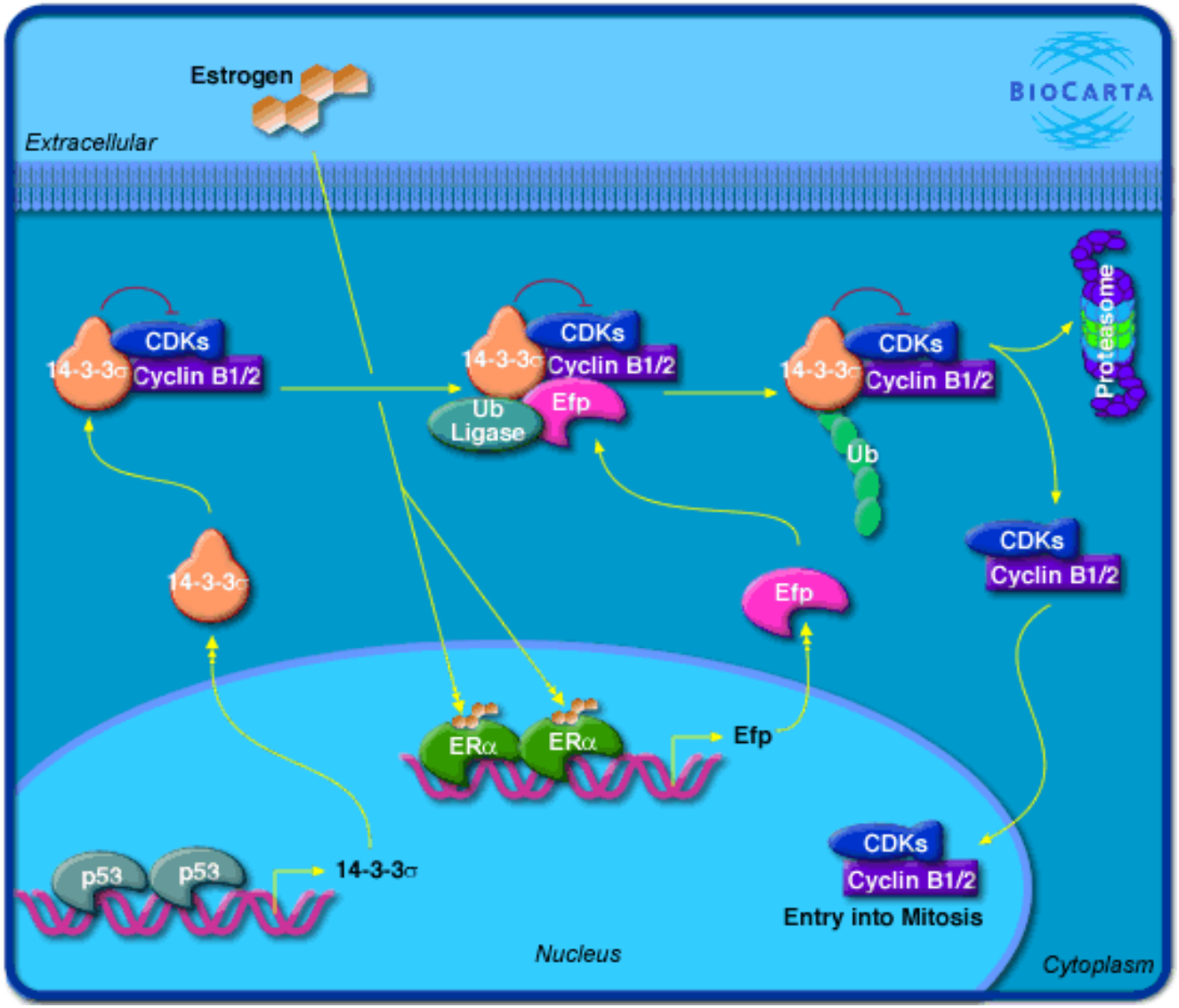
- AOPs are the central element of a toxicological knowledge framework being built to support chemical risk assessment based on mechanistic reasoning.

"Too simplistic"

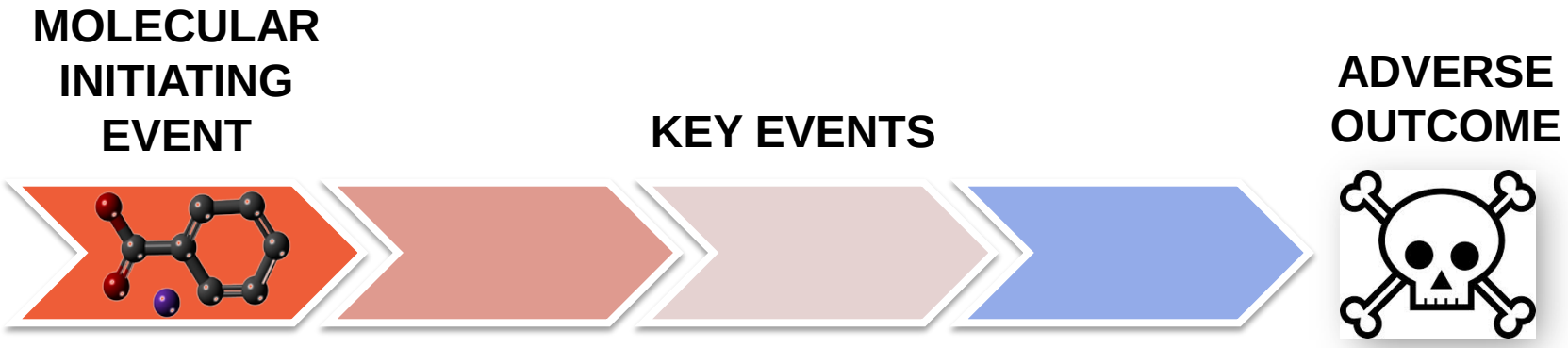
"Nothing new"

*OECD GD138 Developing and Assessing Adverse Outcome Pathways (April 2013)

Estrogen-responsive protein Efp controls cell cycle



Adverse Outcome Pathway (AOP)



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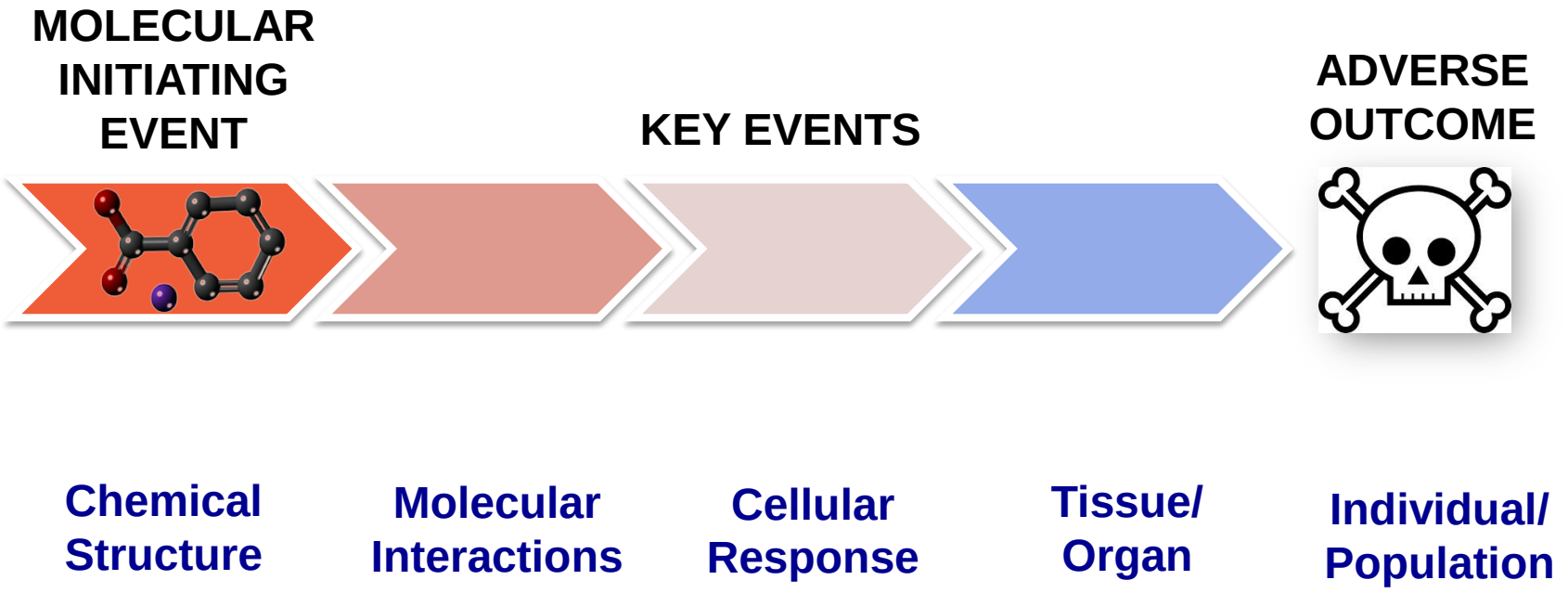
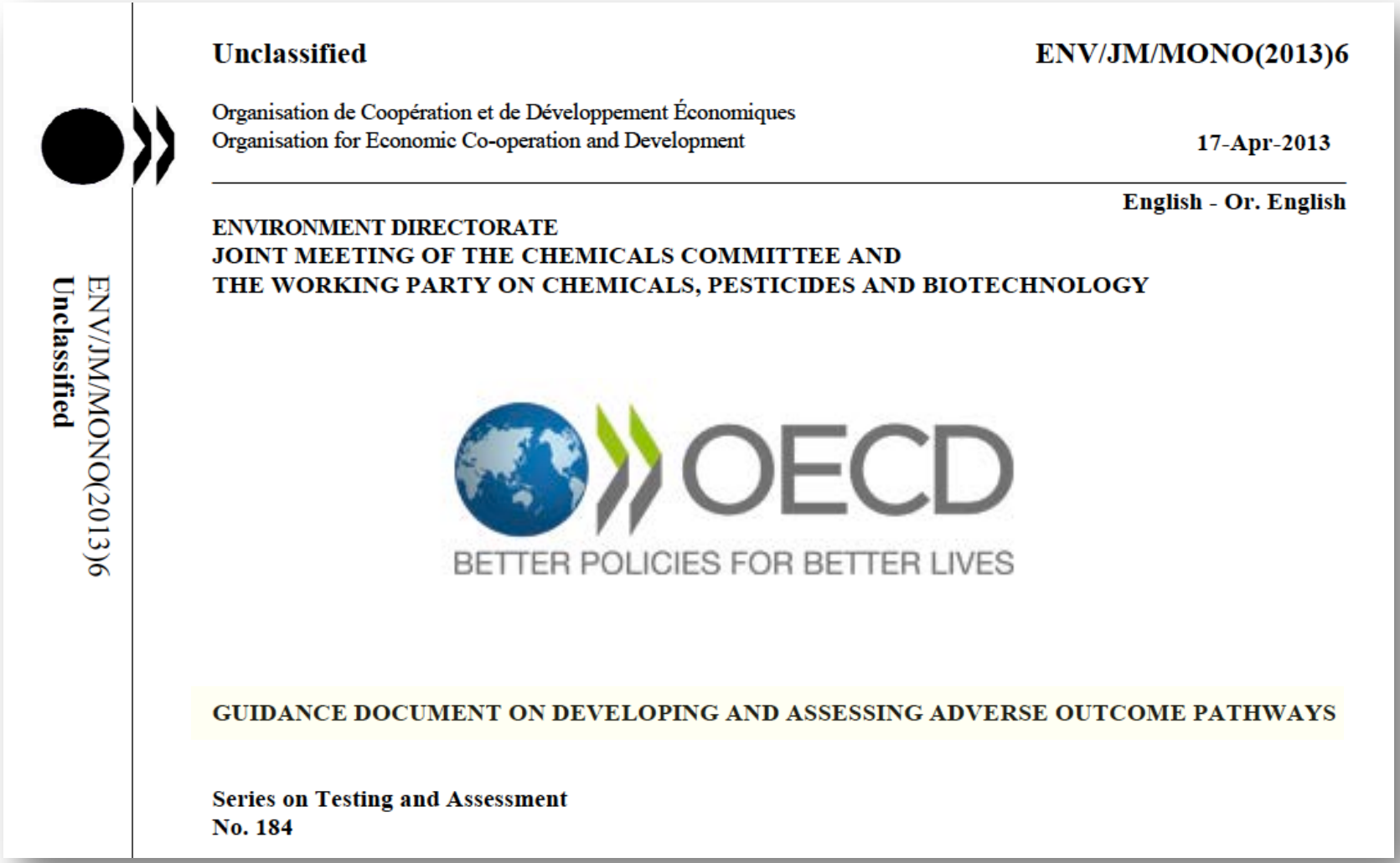


Formalizing the AOP Framework



Unclassified ENV/JM/MONO(2013)6

Unclassified ENV/JM/MONO(2013)6

Organisation de Coopération et de Développement Économiques
Organisation for Economic Co-operation and Development

17-Apr-2013

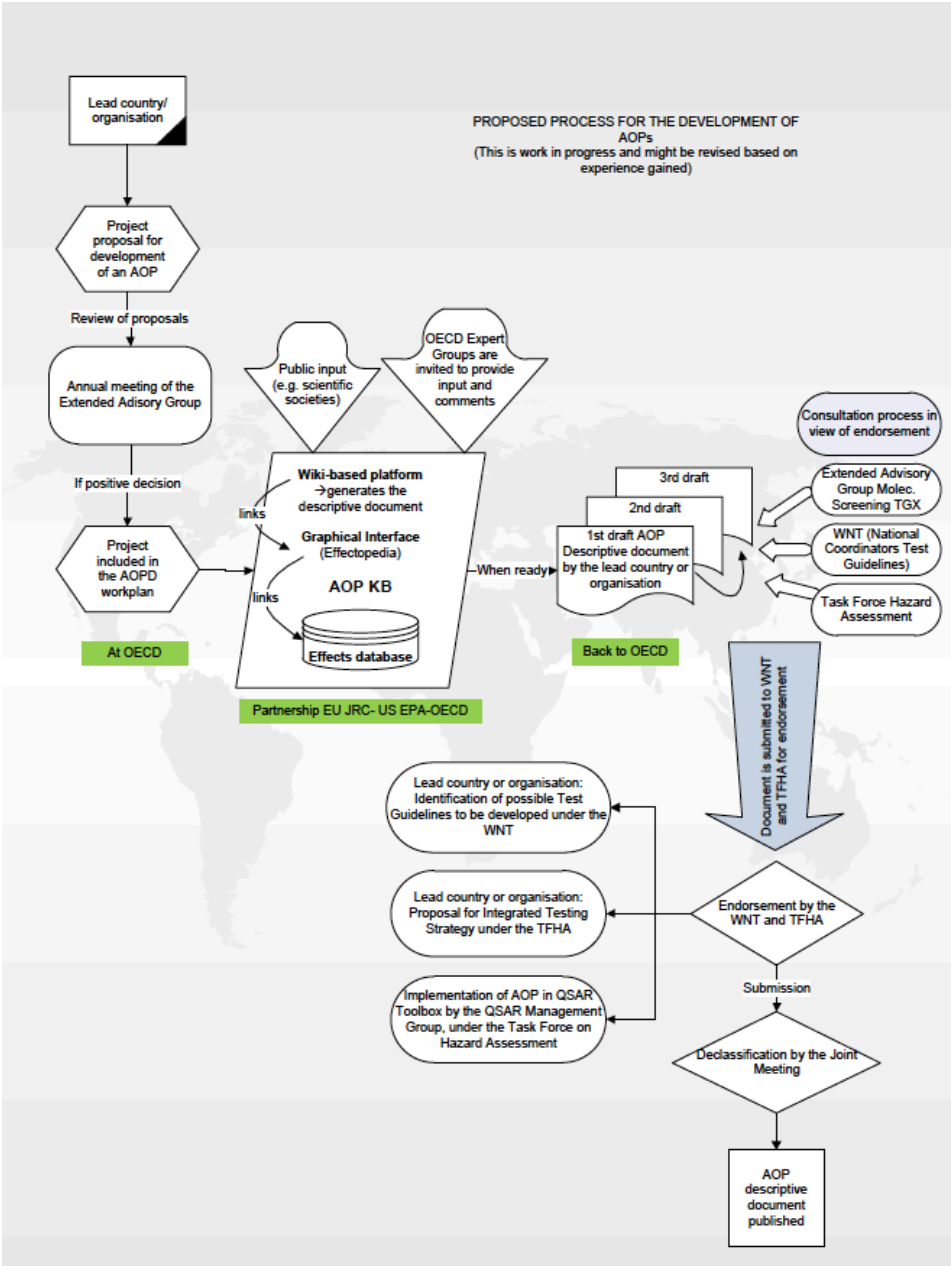
English - Or. English

ENVIRONMENT DIRECTORATE
JOINT MEETING OF THE CHEMICALS COMMITTEE AND
THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY

OECD
BETTER POLICIES FOR BETTER LIVES

GUIDANCE DOCUMENT ON DEVELOPING AND ASSESSING ADVERSE OUTCOME PATHWAYS

Series on Testing and Assessment
No. 184


PROPOSED PROCESS FOR THE DEVELOPMENT OF AOPs
(This is work in progress and might be revised based on experience gained)
Lead country/ organisation
Project proposal for development of an AOP
Review of proposals
Annual meeting of the Extended Adisory Group
If positive decision
Project included in the AOPD workplan
At OECD
Public input (e.g. scientific societies)
OECD Expert Groups are invited to provide input and comments
Wiki-based platform →generates the descriptive document
links
Graphical Interface (Effectopedia)
AOP KB
links
Effects database
Partnership EU JRC- US EPA-OECD
When ready
3rd draft
2nd draft
1st draft AOP Descriptive document by the lead country or organisation
Back to OECD
Consultation process in view of endorsement
Extended Advisory Group Molec. Screening TGX
WNT (National Coordinators Test Guidelines)
Task Force Hazard Assessment
Document is submitted to WNT and TFHA for endorsement
Endorsement by the WNT and TFHA
Submission
Declassification by the Joint Meeting
AOP descriptive document published
Lead country or organisation: Identification of possible Test Guidelines to be developed under the WNT
Lead country or organisation: Proposal for Integrated Testing Strategy under the TFHA
Implementation of AOP in QSAR Toolbox by the QSAR Management Group, under the Task Force on Hazard Assessment

Adverse Outcome Pathway WIKI

OECD GD138

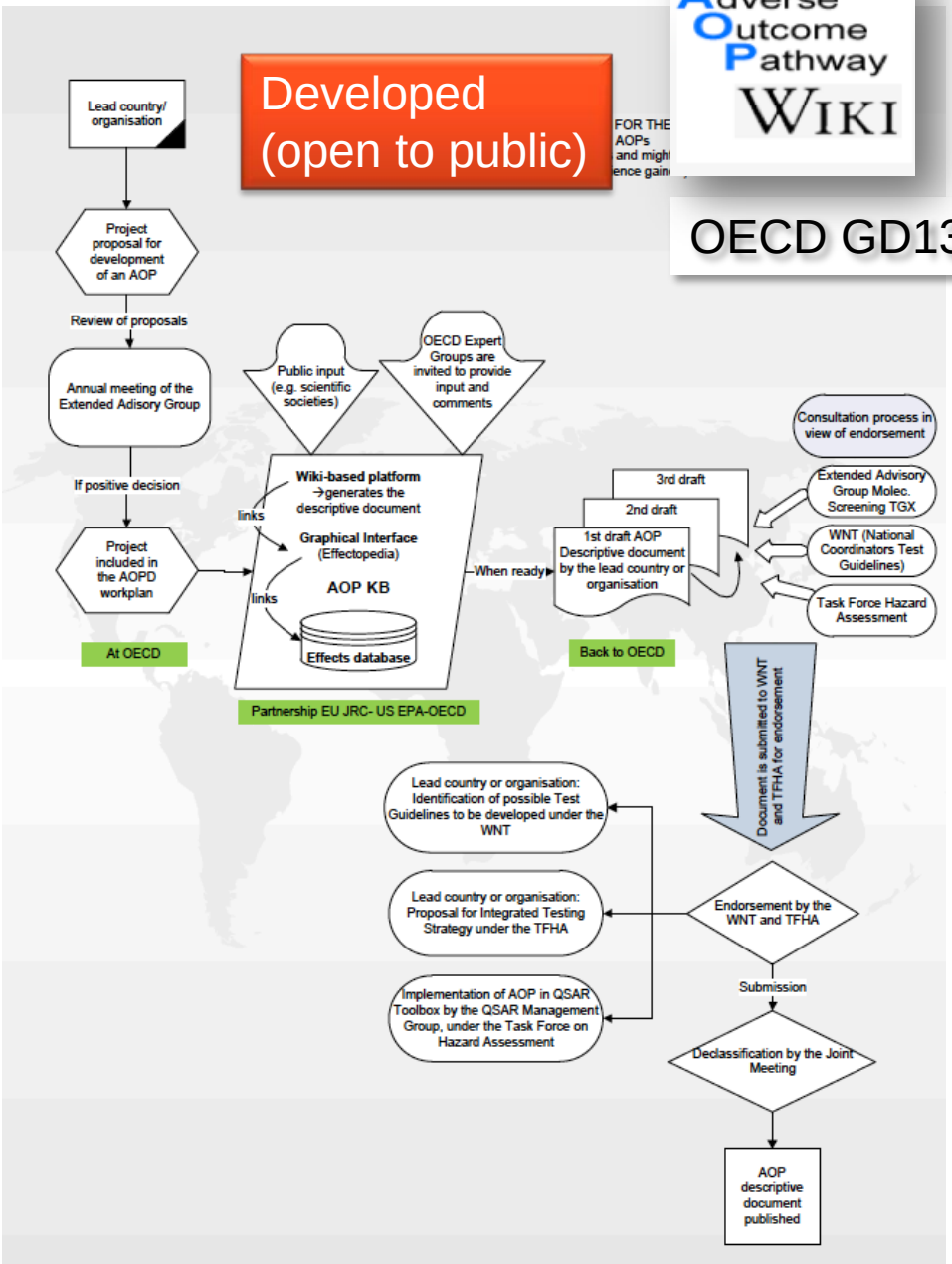
Developed (open to public)

Nominated to OECD

Reviewed By OECD

Endorsed

Published



Example AOPs in the OECD Wiki

- **Dermal Sensitisation Induced by Covalent Binding to Proteins**
- **Heritable Germ Cell-Derived Disease**
- **Neurotoxicity induced by GABAA receptor inhibition**
- **Bile salt export pump inhibition to cholestatic liver injury**
- **Respiratory Sensitisation Induced by Covalent Binding to Proteins**
- **Embryonic Vascular Disruption and Developmental Defects**
- **Aryl Hydrocarbon Receptor (AHR) Adverse Outcome Pathway for a Range of Species-Specific Effects**
- **Mutagenic mode of action for chemical carcinogens**
- **Hepatotoxicity due to 2,4,6-trinitrotoluene AOP on Energy Metabolism affected by 2,6-Dinitroluene**

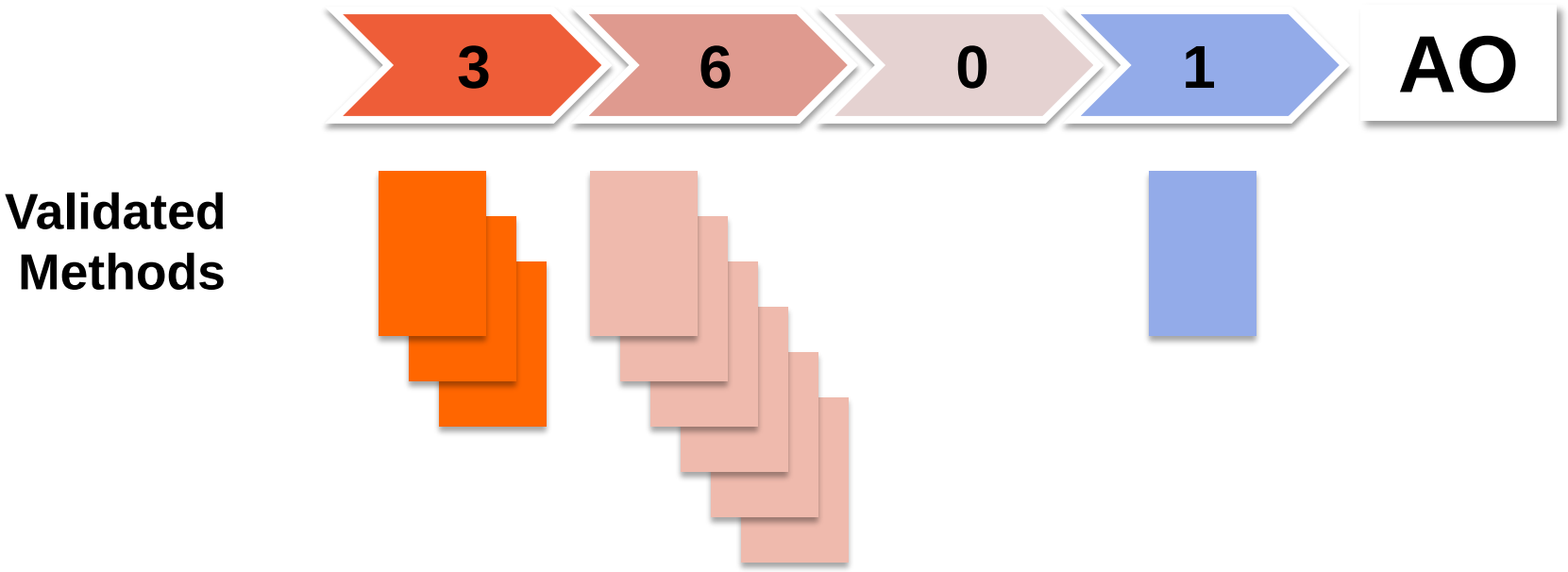
AOP Utilization

- Inform data gaps for development of *in vitro, in silico, in vitro* assays

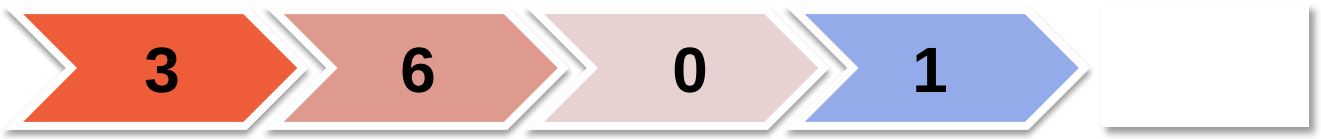
- Assist in the development of Hazard Identification and Risk Assessment :
  - Tiered testing approaches (screening)
  - Integrated Testing and Decision Strategies (ITDS)

AOP Utilization

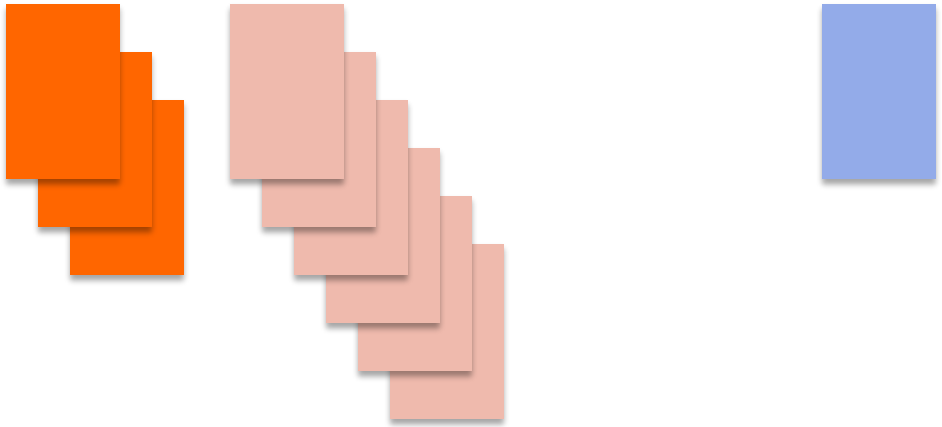
AOP Utilization



Integrated Testing and Decision Strategies (ITDS)



Validated Methods

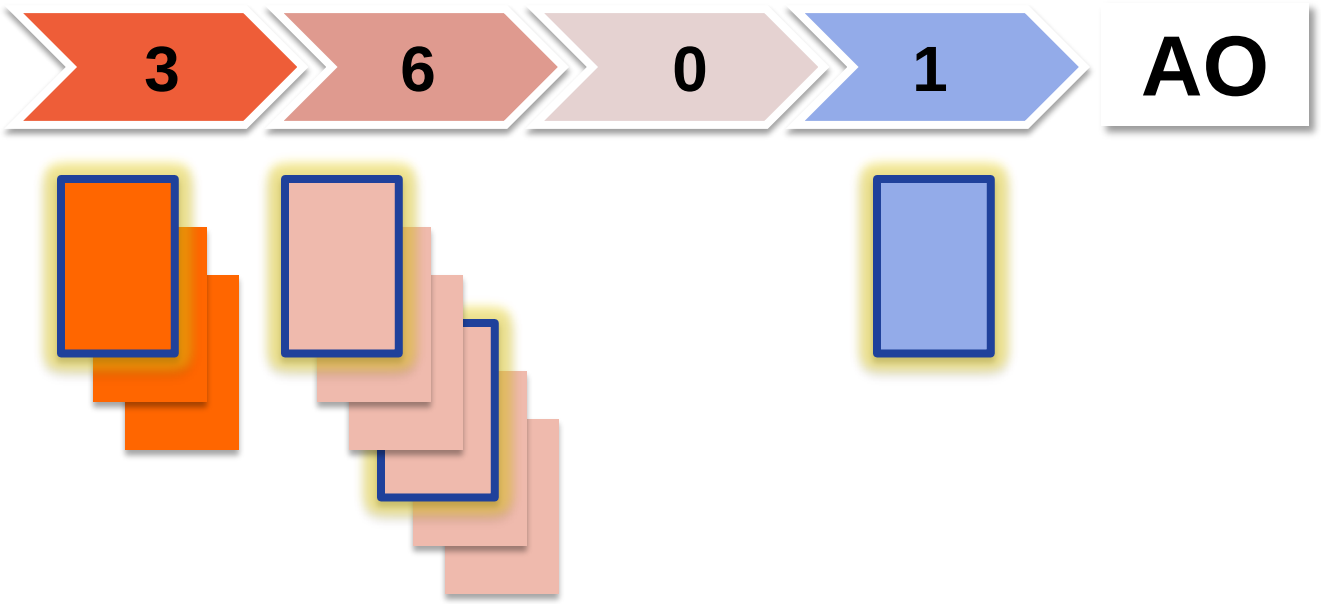


Data Integration

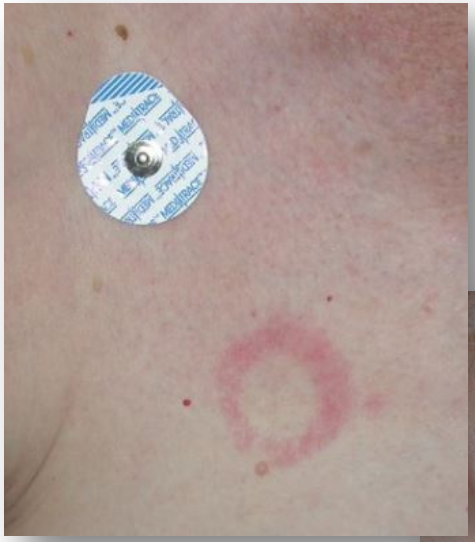
$$I(X;Y) = \sum_{y \in Y} \sum_{x \in X} p(x,y) \log \left(\frac{p(x,y)}{p(x)\,p(y)} \right)$$

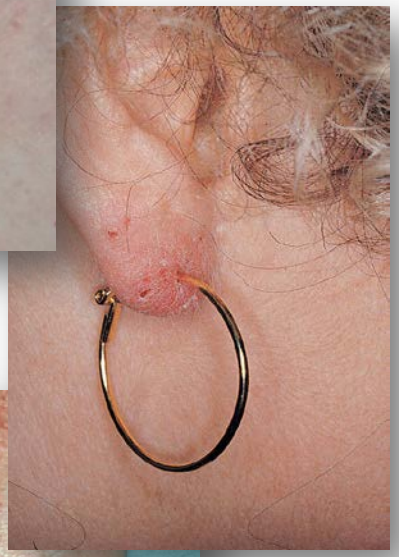
***p*(AO)**

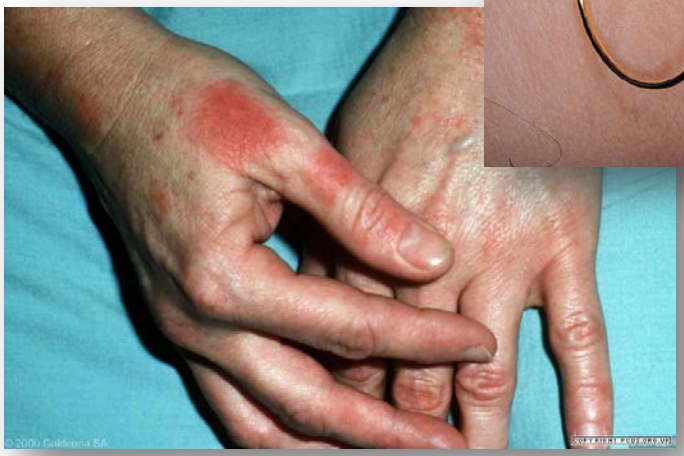
Integrated Testing and Decision Strategies (ITDS)



Skin Sensitization Testing







- Public Health
- Animal Welfare
- Scientific Perspective

Skin Sensitization Pathway

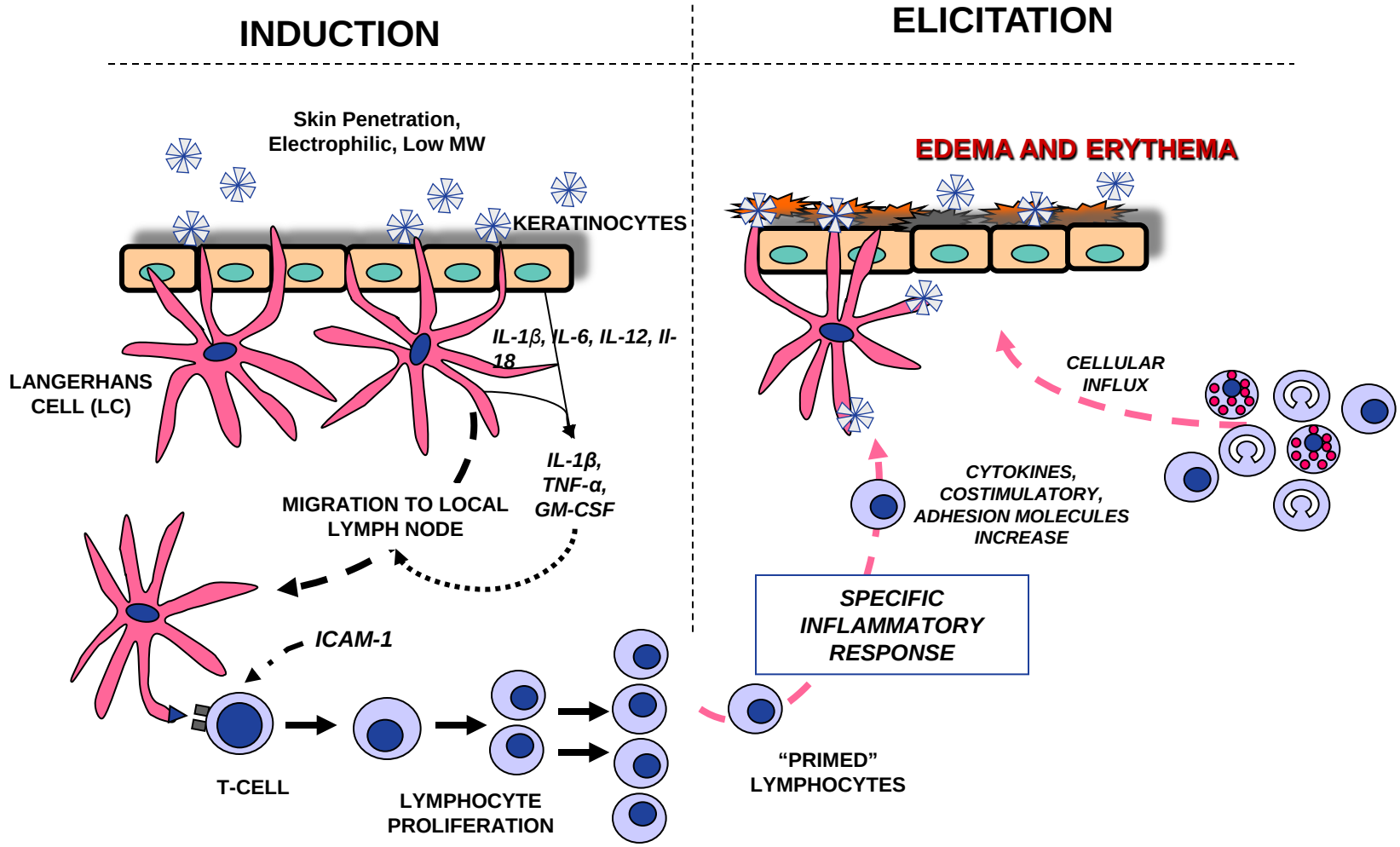
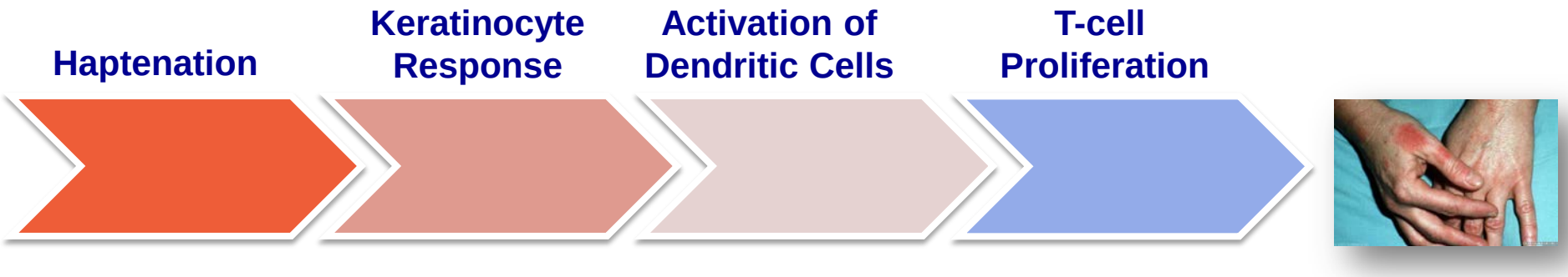


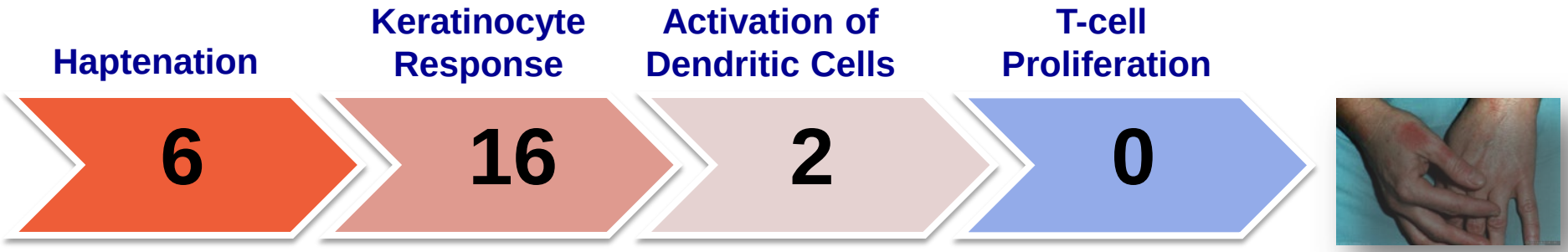
*Illustration by D. Sailstad

Skin Sensitization AOP



Key Events	Experimental Support	Strength of Evidence
Key Event 1 (initial event)	Site of action proteins (see Karlberg et al., 2008; Wong and Liebler, 2008). Covalent binding at cysteine and/or lysine (see Roberts and Natsch, 2009; Schwöbel et al., 2011).	**Strong**; well-accepted mode of toxic action associated with skin sensitisation with 100s of chemicals evaluated for binding in quantitative endpoints.

Skin Sensitization AOP



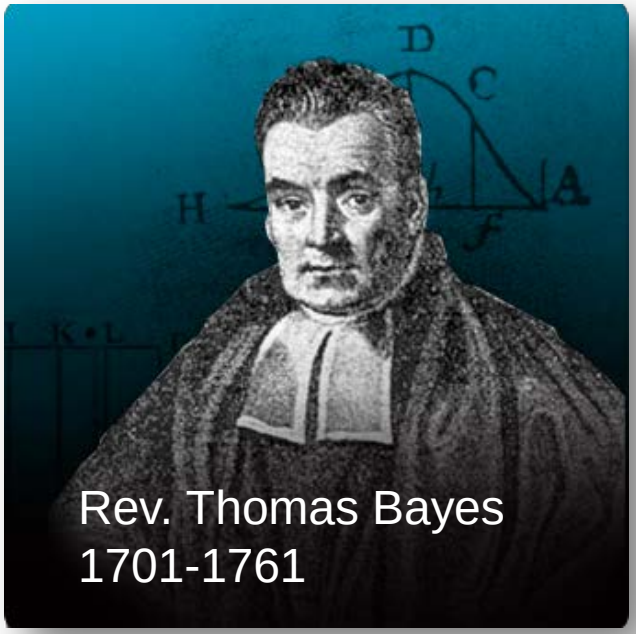
- **Number of *in vitro* methods currently <u>undergoing validation</u>**
- ***All for use as part of an integrated testing strategy***

Bayesian Networks

$$P(A \mid B) = \frac{P(B \mid A)P(A)}{P(B)}$$

A way at arriving at statistical likelihood based on partial information.

Bayesian networks provide a coherent probabilistic framework for reasoning and guiding decisions on the classification of a substance or the need for additional testing.



Rev. Thomas Bayes
1701-1761

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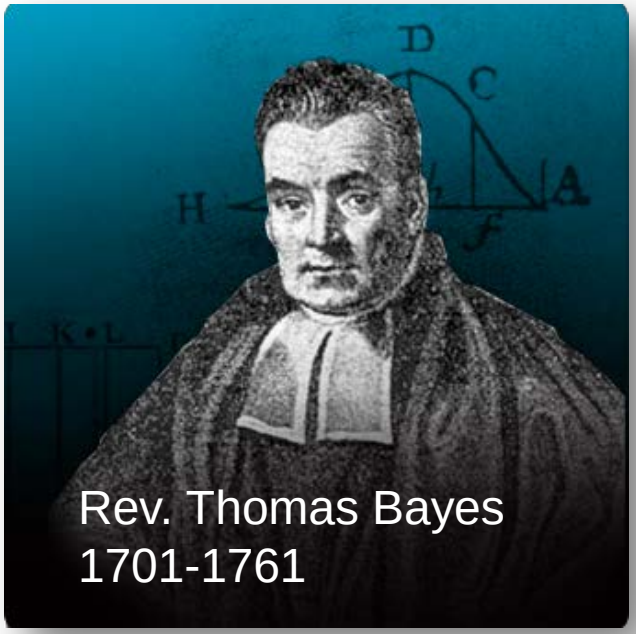

Rev. Thomas Bayes
1701-1761

"*The most remarkable feature of Bayes's theorem is that it had no practical applications in his lifetime without computers to do the necessary calculations*".

- Bill Bryson

Q1


LIBERTY
IN GOD WE TRUST
UNITED STATES OF AMERICA
QUARTER DOLLAR

Q2


LIBERTY
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Q3


LIBERTY
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LIBERTY
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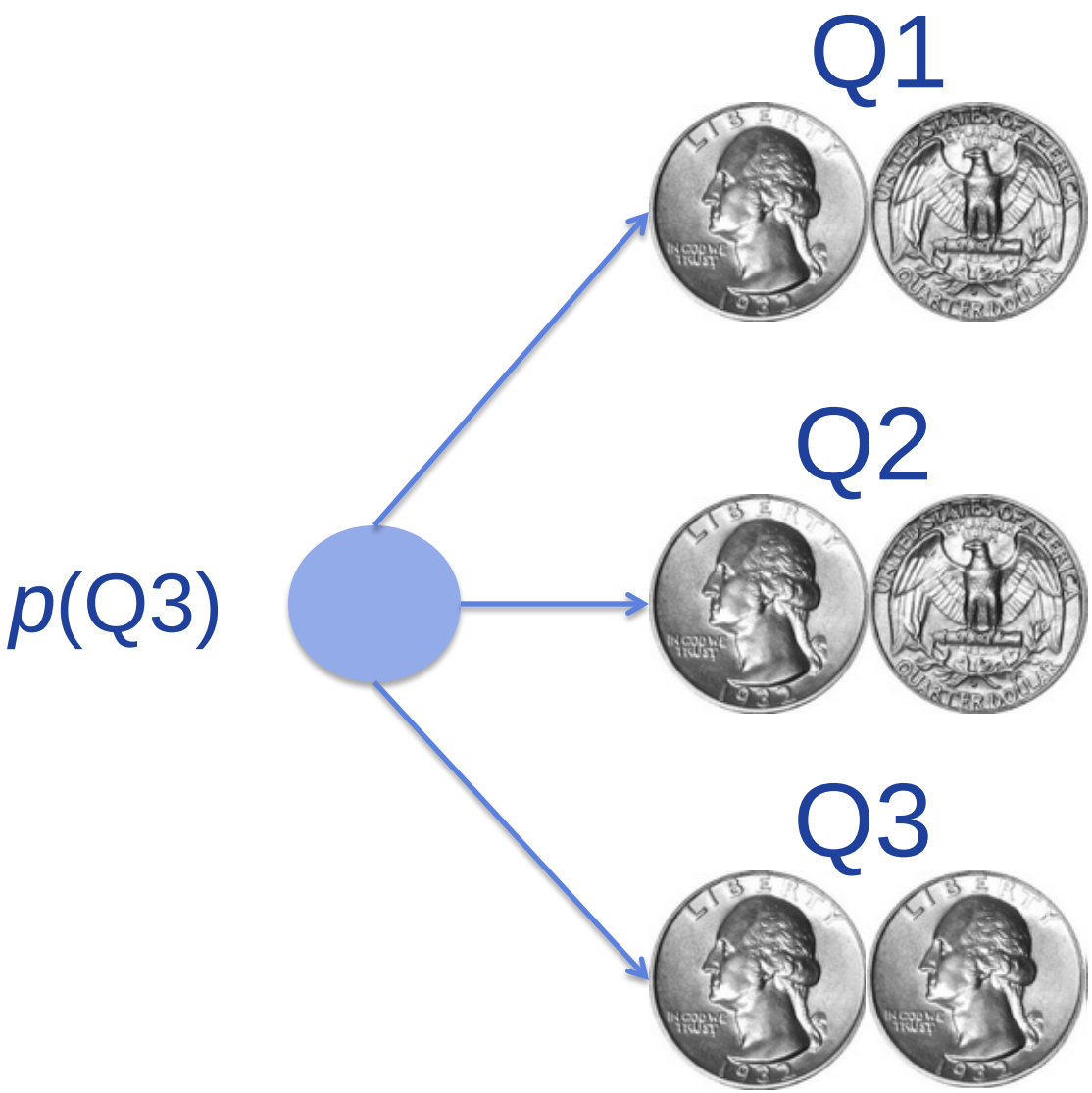
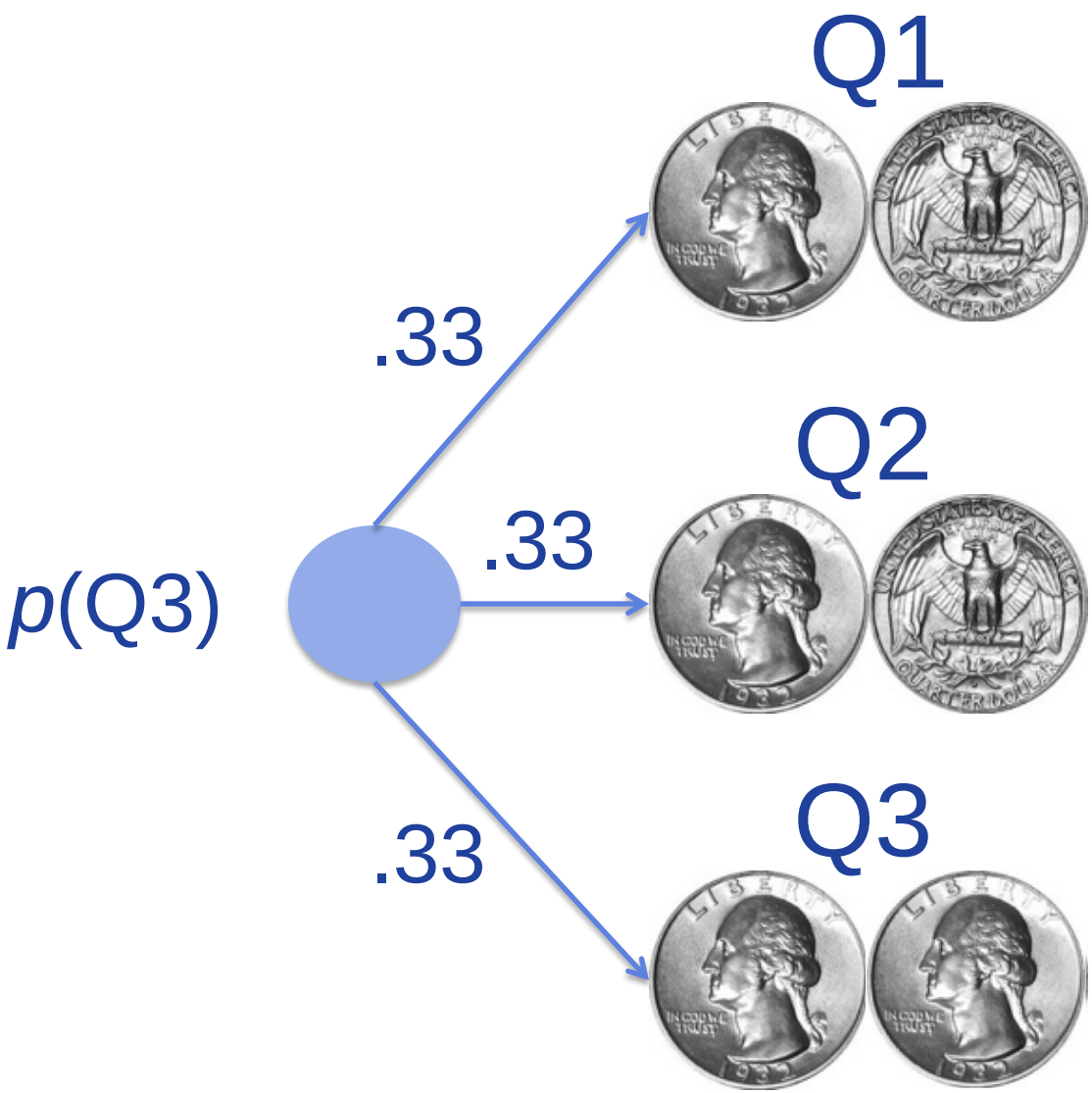
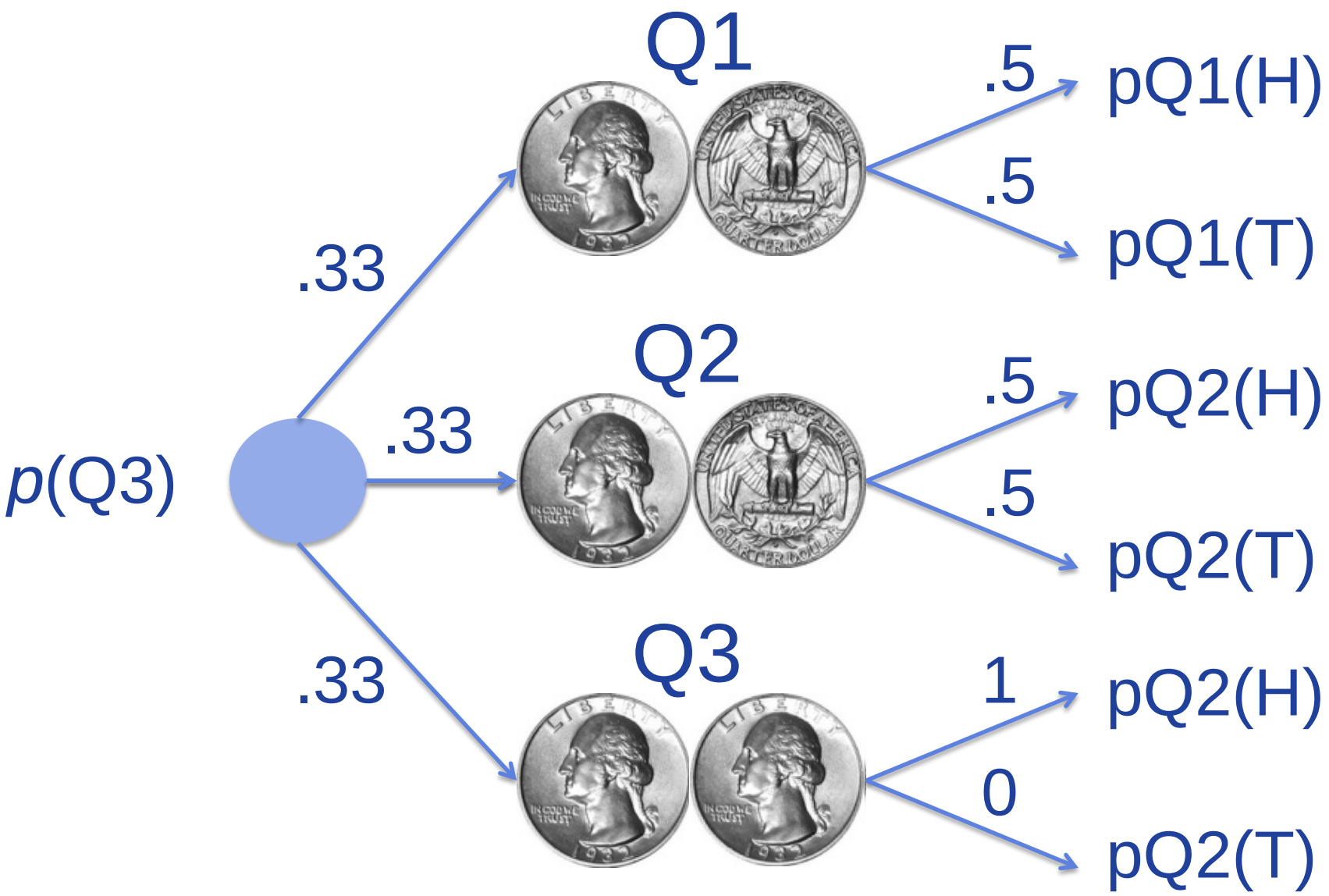
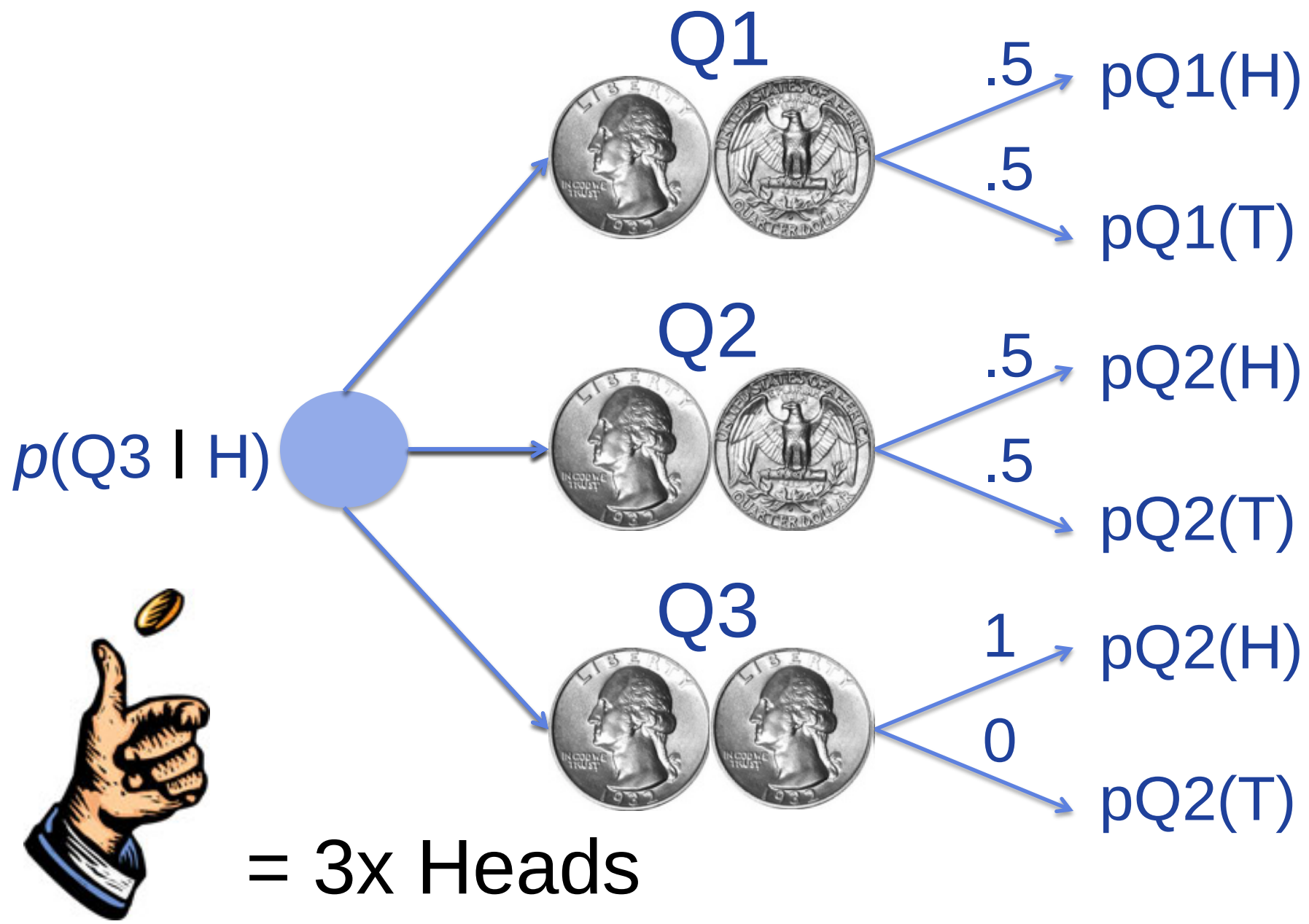
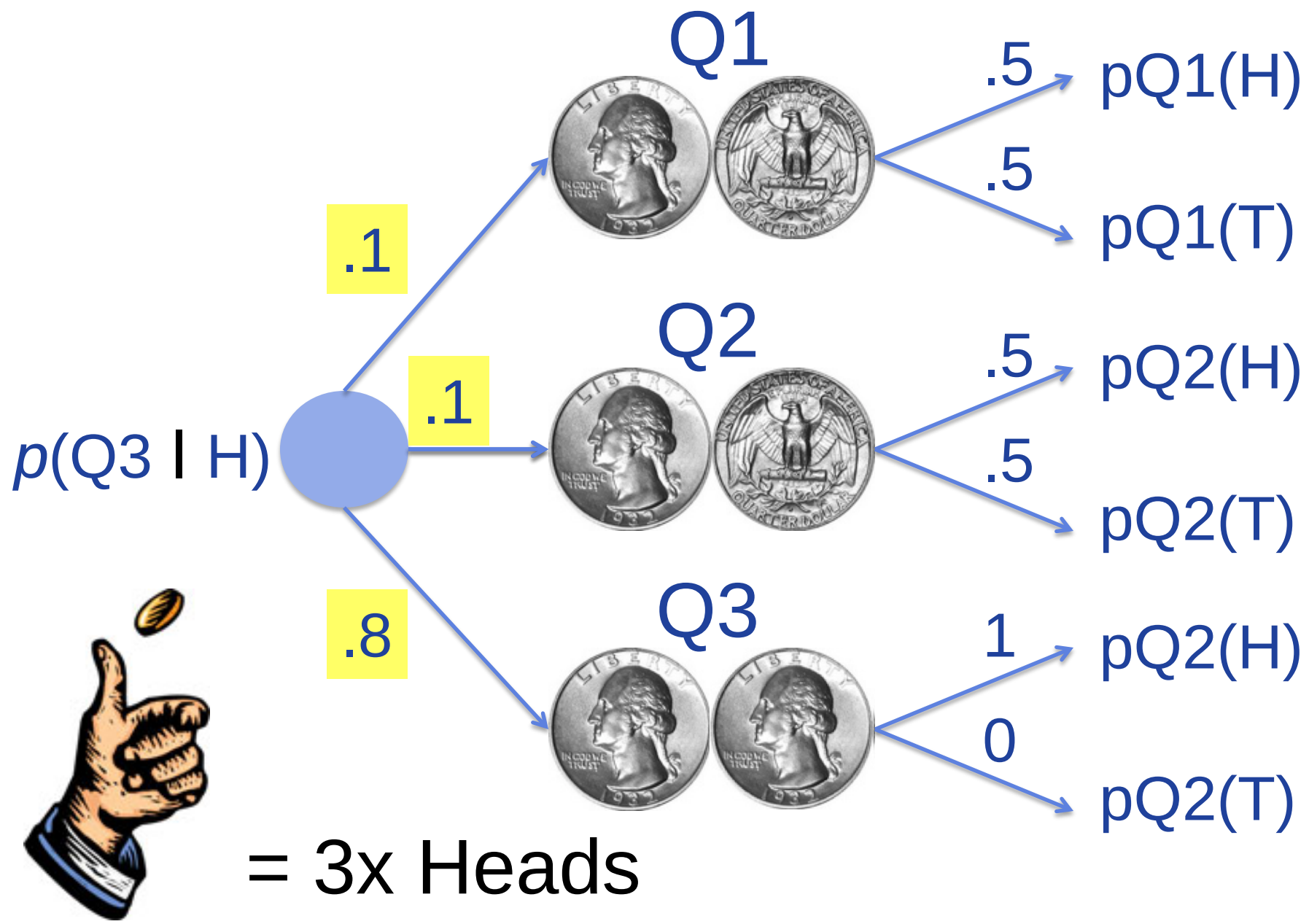

Q1
Q2
Q3
p(Q3)


Q1
Q2
Q3
.33
.33
.33
p(Q3)


Q1
Q2
Q3
p(Q3)
.33
.33
.33
.5
.5
.5
.5
1
0
pQ1(H)
pQ1(T)
pQ2(H)
pQ2(T)
pQ2(H)
pQ2(T)

= 3x Heads


Q1
.5
pQ1(H)
.5
pQ1(T)
Q2
.5
pQ2(H)
.5
pQ2(T)
p(Q3 | H)
Q3
1
pQ2(H)
0
pQ2(T)
= 3x Heads


Q1
Q2
Q3
.1
.1
.8
p(Q3 | H)
.5
.5
pQ1(H)
pQ1(T)
.5
.5
pQ2(H)
pQ2(T)
1
0
pQ2(H)
pQ2(T)
= 3x Heads

Research Article

Journal of Applied Toxicology

Received: 14 January 2013, Revised: 4 February 2013, Accepted: 4 February 2013 Published online in Wiley Online Library

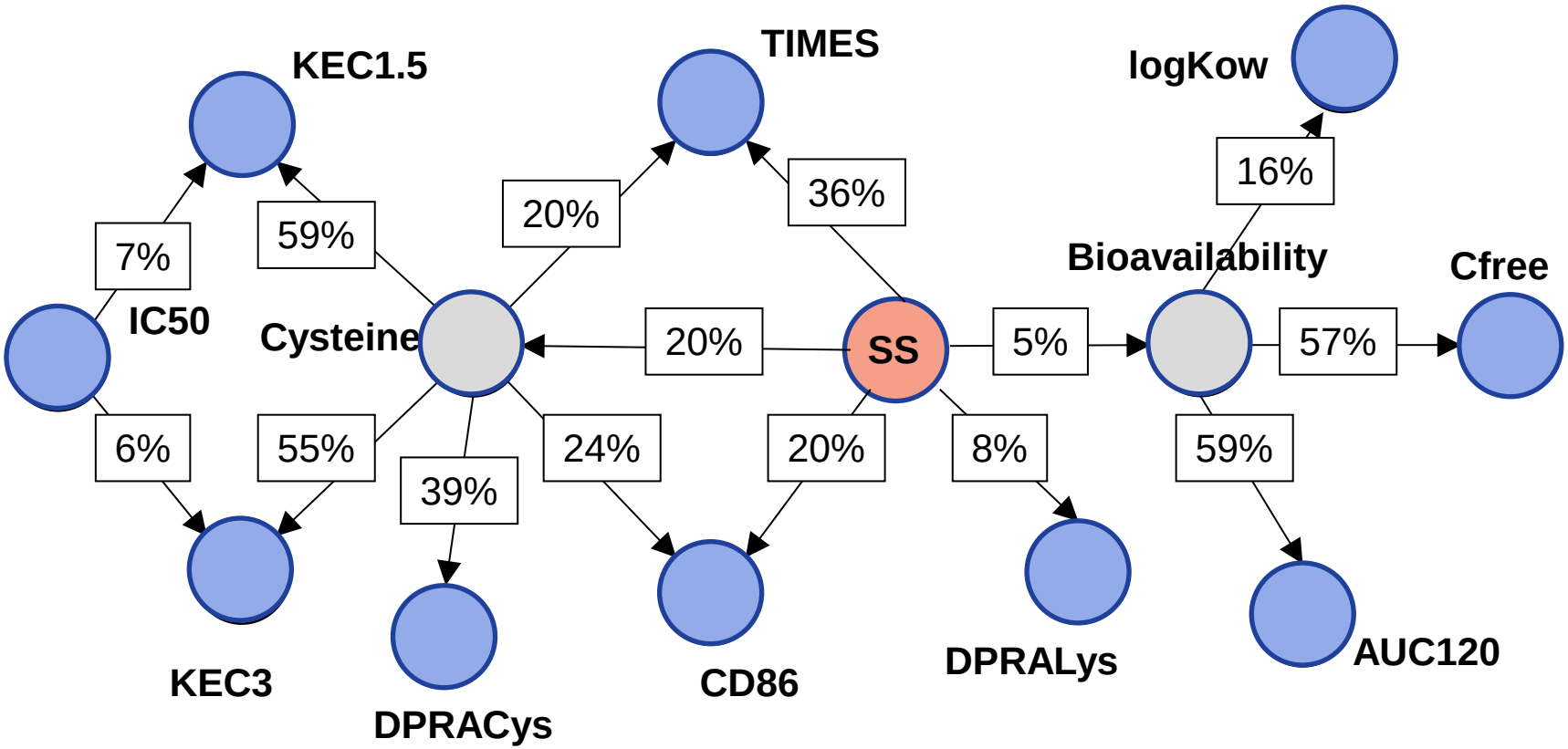
(wileyonlinelibrary.com) DOI 10.1002/jat.2869

Bayesian integrated testing strategy to assess skin sensitization potency: from theory to practice

Joanna Jaworska[a]*, Yuri Dancik[a], Petra Kern[a], Frank Gerberick[b] and Andreas Natsch[c]

ABSTRACT: Frameworks to predict *in vivo* effects by integration of *in vitro*, *in silico* and *in chemico* information using mechanistic insight are needed to meet the challenges of 21st century toxicology. Expert-based approaches that qualitatively integrate multifaceted data are practiced under the term 'weight of evidence', whereas quantitative approaches remain rare. To address this gap we previously developed a methodology to design an Integrated Testing Strategy (ITS) in the form of a Bayesian Network (BN). This study follows up on our proof of concept work and presents an updated ITS to assess skin sensitization

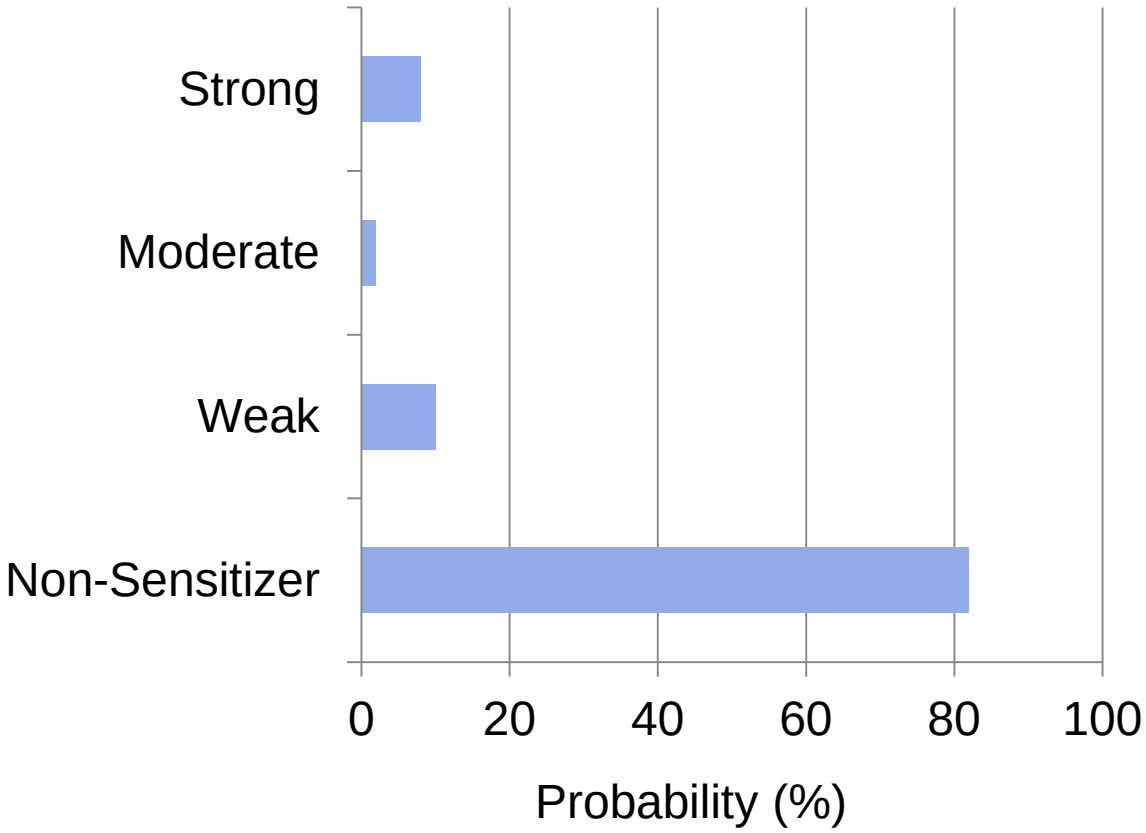
P&G Procter & Gamble



External validation: 86% correct for potency, 95% for hazard

Jaworska et al. 2013

Output is a probability distribution



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Open Source software

ALTEX Online first
published March 31, 2014
http://dx.doi.org/10.14573/altex.1310151

SHORT COMMUNICATION

Open Source Software Implementation of an Integrated Testing Strategy for Skin Sensitization Potency Based on a Bayesian Network

Jason R. Pirone[1], Marjolein Smith[1], Nicole C. Kleinstreuer[2], Thomas A. Burns[2], Judy Strickland[2], Yuri Dancik[4], Richard Morris[1], Lori A. Rinckel[2], Warren Casey[3], and Joanna S. Jaworska[4]

[1]Social and Scientific Systems, Inc., Durham, NC, USA; [2]Integrated Laboratory Systems, Morrisville, NC, USA; [3]National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods, Division of the National Toxicology Program, National Institute of Environmental Health Sciences, Research Triangle Park, NC, USA; [4]Procter & Gamble NV, Strombeek-Bever, Belgium

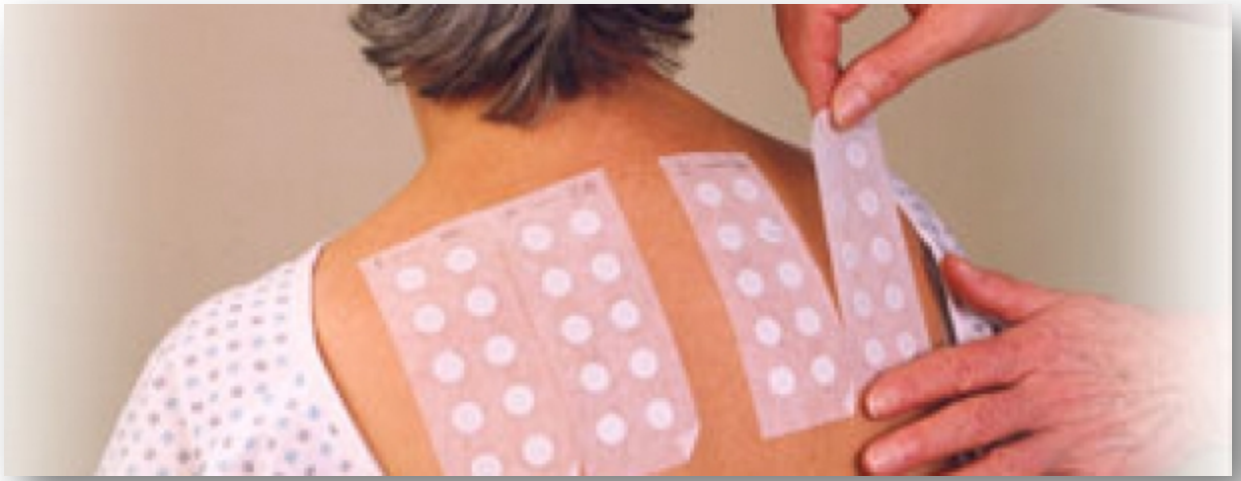
http://ntp.niehs.nih.gov/go/its

Regulatory Applicability of AOPs

- Pilot Project: Validate IDTS for use by regulatory agencies using a variety of computational approaches to determine the sensitizing potential of chemicals.

Skin Sensitization Tests

- Buehler Test and Guinea Pig Maximization Test
- Local Lymph Node Assay (LLNA), Mouse
- Human skin patch tests



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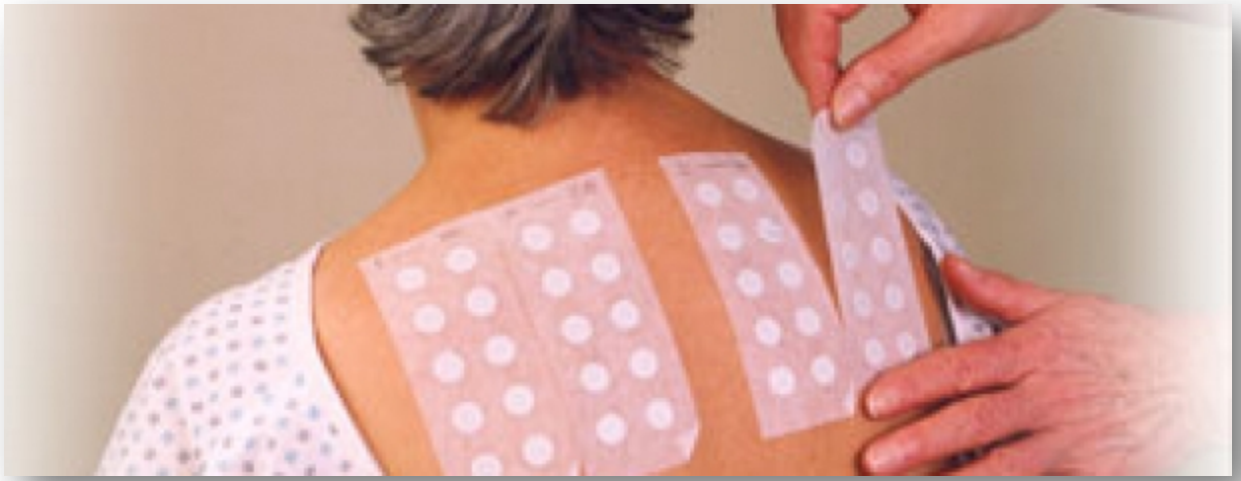
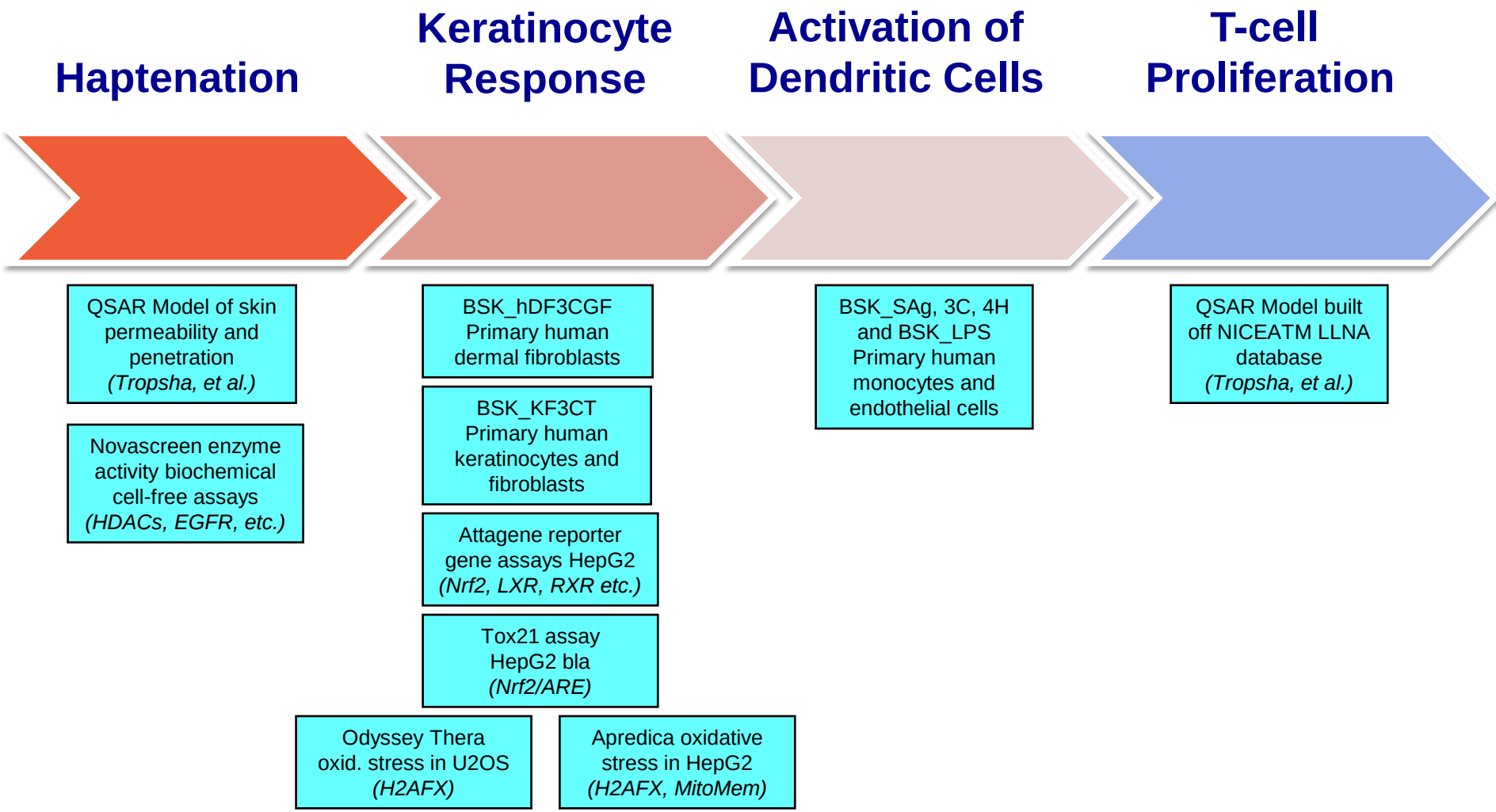


- Yes / No or Potency (and Incidence?)

Regulatory Applicability of AOPs

- Pilot Project: Validate IDTS using a variety of computational approaches to classify chemicals as sensitizers / non sensitizers, using LLNA as the reference (current EPA OPP Requirements)

ToxCast/Tox21 Methods



NATURE BIOTECHNOLOGY VOLUME 32 NUMBER 6 JUNE 2014 ARTICLES

nature biotechnology

Phenotypic screening of the ToxCast chemical library to classify toxic and therapeutic mechanisms

Nicole C Kleinstreuer[1], Jian Yang[2], Ellen L Berg[2], Thomas B Knudsen[1], Ann M Richard[1], Matthew T Martin[1], David M Reif[1], Richard S Judson[1], Mark Polokoff[2], David J Dix[1], Robert J Kavlock[1] & Keith A Houck[1]

Addressing the safety aspects of drugs and environmental chemicals has historically been undertaken through animal testing. However, the quantity of chemicals in need of assessment and the challenges of species extrapolation require the development of alternative approaches. Our approach, the US Environmental Protection Agency's ToxCast program, utilizes a large suite of *in vitro* and model organism assays to interrogate important chemical libraries and computationally analyze bioactivity profiles. Here we evaluated one component of the ToxCast program, the use of primary human cell systems, by screening for chemicals that disrupt physiologically important pathways. Chemical-response signatures for 87 endpoints covering molecular functions relevant to toxic and therapeutic pathways were generated in eight cell systems for 641 environmental chemicals and 135 reference pharmaceuticals and failed drugs. Computational clustering of the profiling data provided insights into the polypharmacology and potential off-target effects for many chemicals that have limited or no toxicity information. The endpoints measured can be closely linked to *in vivo* outcomes, such as the upregulation of tissue factor in endothelial cell systems by compounds linked to the risk of thrombosis *in vivo*. Our results demonstrate that assaying complex biological pathways in primary human cells can identify potential chemical targets, toxicological liabilities and mechanisms useful for elucidating adverse outcome pathways.

Workshop

September 3-5, 2014
William H. Natcher Conference Center
National Institutes of Health
Bethesda, Maryland, USA